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The Effect of Soaking Eggs in Natural Plant Extracts on Embryogenesis, Hatching, and Survival of Pawas Fish Larvae (*Osteochilus Hasselti* CV)

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Abstract

The pawas fish (Osteochilus hasselti CV) is an economically important freshwater fish commodity, but the availability of quality fry remains a constraint in aquaculture activities. This study aims to analyze the effect of egg immersion using various natural plant extracts on embryogenesis development, hatching time, abnormalities, and pawas fish larvae survival. The study was conducted at the Fish Breeding and Breeding Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, from August to October 2025 using a completely randomized design with five treatments and three replicates, namely control without soaking, pineapple extract, star fruit extract, cherry leaf extract, and papaya leaf extract. Fertilized eggs were soaked for 4 minutes and then incubated. The results showed that soaking the eggs in natural plant extracts affected the observed parameters. The best treatment was obtained by soaking the eggs in 4 mL/L cherry leaf extract for 20 hours and 14 minutes, which resulted in the lowest number of abnormal larvae and the highest survival rate of 64.03%.

Keywords: Pawas Fish, Natural Plant Extracts, Embryogenesis, Survival Rate

1. Introduction

Pawas fish (Osteochilus hasselti CV) is a freshwater fish native to Indonesia that is widely loved by the community (Rochmatin *et al*., 2014) [13]. Pawas fish (Osteochilus hasselti CV) is one of the economically important fish species found in the waters of the Kampar River, Riau. Pawas fish farming has grown and developed in fish farming activities, but the supply of seeds for these farming activities is still an obstacle that is always encountered. In addition, the availability of sufficient seeds, both in terms of quality and quantity, is a determining factor in the success of a farming business (Sukendi *et al* 2020) [20]. However, the success of artificial spawning is highly dependent on the quality of eggs produced by prospective broodstock, because good quality eggs will produce good quality fry in large quantities (Sukendi *et al* 2016) [17]. Cultivation is one solution to increase fish catch (Sukendi *et al* 2021) [18]. To overcome this problem, eggs can be soaked in a solution containing several types of natural plants that can remove this sticky property. Some plants that can be used are pineapple (Ananas comosus), star fruit (Belimbing wuluh), cherry leaves (Mutingia calabura), and papaya leaves (Carica papaya). In addition, another problem faced after the hatching of pawas fish is the high mortality rate during the larval stage. This occurs when the yolk sac is depleted and the fish are unable to find suitable food. External feeding must be carried out when the yolk sac is depleted so that the larvae have sufficient nutrients (Mariska *et al*. 2013). Therefore, the purpose of this paper is to find the best plant species to increase pawas fish seed production and overcome the obstacles to pawas fish mortality.

2. Materials and Methods

This study was conducted at the Fish Breeding and Improvement Laboratory (PPI), Faculty of Fisheries and Marine Sciences, University of Riau, from August to October 2025. The materials used in this study were mature pawas fish broodstock (TKG IV), distilled water, 0.9% NaCl solution, 70% alcohol, fertilization solution, and silkworms. The treatments used in this study were pineapple, star fruit, cherry leaves, and papaya leaves. The equipment used in this study included buckets, basins, 1 ml and 3 ml syringes, an Olympus binocular microscope, catheters, scales, aerators and their installations, DO meters, pH meters, thermometers, chicken feathers, hatching tanks, aquariums, cameras, scoops and nets, a ruler, small bowls, small towels,

sieves, styrofoam, a tally counter, and a set of surgical instruments. The treatments in this study consisted of treatment P0 (control treatment without immersion in natural solution), P1 (immersion treatment with a dose of pineapple solution of 1 ml/liter of water), P2 (treatment with a dose of 4.0 ml/liter of star fruit solution), P3 (treatment with a dose of 4.0 ml/liter of cherry leaf solution), and P4 (treatment with a dose of 4.0 ml/liter of papaya solution). The design used was a completely randomized design with 5 treatment levels and 3 replicates, resulting in 15 treatment units. The fertilized eggs were immersed for 4 minutes in a 1-liter basin with a volume of 1 liter of water for each predetermined dose treatment. The soaked eggs were then transferred to an incubation container to observe the measured parameters. The parameters observed and measured in this study were as follows: embryogenesis development, hatching time, pigmentation development, fin morphology development, abnormalities, and survival rate.

3. Results and Discussion

Development of pawas fish embryogenesis The results of observations on the development of pawas fish larvae embryogenesis for each treatment can be seen in Table 1.

Table 1: Development of pawas fish larvae embryogenesis from each treatment

Embryogenesis Phase	Observation Time									
	P0	P1		P2		P3			P4	
	Hour	Hour	Minute	Hour	Minute	Hour	Minute		Hour	Minute
Early Morula	07	15	07	16	07	18	07	19	07	18
Middle Morula	07	47	07	46	07	44	07	43	07	42
Final Morula	08	29	08	28	08	27	08	27	08	26
Early Blastula	08	56	08	57	08	57	08	58	08	57
Middle Blastula	09	56	09	57	09	57	09	58	08	57
Final Blastula	11	36	11	36	11	37	11	38	11	37
Early Gastrula	11	51	11	51	11	52	11	53	11	52
Middle Gastrula	14	54	14	54	14	53	14	55	14	53
Final Gastrula	00	45	00	44	00	45	00	47	00	46
Organogenesis	01	35	01	34	01	35	01	37	01	36
Hatching	02	50	02	50	02	53	02	55	02	54

Table 1 shows that the earliest morula phase occurred in treatments P1 and P2 at 07:18 minutes after fertilization, while the latest occurred in treatment P3 at 07:19 minutes after fertilization. Morula is the process of cell division above 32 cells or 64 daughter cells. The daughter cells produced from the division of the zygote are called blastomeres (Windarti *et al*., 2017) [19]. According to Sukendi (2003) [16], morula cells are divided into protrusions containing many yellow egg curls. The protoplasm layer is concentrated between the yolk and the cell mass (perioblast). The final morula stage occurs at 6 hours. The earliest blastula phase is at P1, occurring at 08:56 minutes after fertilization, and the latest is at P3, occurring at 11:38 minutes after fertilization. According to Sukra (2000) in Rahayu (2013) [12], blastula is the process of morula development into blastula. At this stage, the blastomer cells in the morula divide several times, becoming smaller and smaller, and towards the end of the division, some of the blastomer cells under the morula fall off, leaving a space that was originally dense, called the blastosul or blastocoels. The earliest gastrula phase occurred at P1, at 11:51 a.m., and the longest at P3, which is at 00:47 minutes. According to Sukendi (2003) [16], the gastrula stage occurs 3 hours after the blastocoels are formed, where the cells in the thickest marginal part begin to form indentations (invaginate) in the cytoplasm or egg yolk, which is the beginning of the gastrula stage. Effendie (2002) [2] states that along with the completion of the gastrulation process, the formation of organs (organogenesis) has actually begun, preceded by a kind of tube formation by the epidermal, neural, mesoderm, and endoderm tissues. The neural tube is formed by the sinking of the neural groove originating from the ectoderm layer. Organs formed from neural tissue include the brain, ganglia, and eyes. The embryo then enters the phase of organ formation.

Hatching Time of Pawas Fish Eggs

The results of the table showing the hatching time of pawas fish eggs from each treatment can be seen in Table 2.

Table 2: Incubation period of pawas fish eggs from each treatment

Treatment	Hatching Time (Hours/Minutes)
P0 (Control)	20 Hour 5 Minutes
P1 (Pineapple Stalk)	20 Hour 7 Minutes
P2 (Star Fruit)	20 Hour 11 Minutes
P3 (Cherry Leaf)	20 Hour 14 Minutes
P4 (Papaya Leaf)	20 Hour 12 Minutes

Table 2 shows that the fastest hatching time in P1 was 20 hours and 5 minutes, and the longest hatching time in P3 was 20 hours and 14 minutes. The hatching rate is the percentage of eggs that successfully hatch compared to the initial number of eggs (Fariedah, 2018). Hatching power is an important factor in the hatchery cultivation process, because high hatching power produces many larvae so that the seed supply and production processes run well. According to Nugraha *et al*. (2012) [11], the hatching process occurs due to the mechanical and enzymatic work of the embryo, which requires a lot of energy, so adequate recirculation is needed. Factors that influence egg development during incubation, aside from oxygen, include the stability of water movement. Slow water movement results in little movement of the eggs, causing a slow metabolic process that prolongs the hatching period and can create an ideal environment for fungal growth, inhibiting egg development. Conversely, excessively fast water movement can accelerate the metabolic process excessively, causing abnormalities and even killing the eggs or larvae. Hatching rate is influenced by several factors such as temperature, oxygen, DO, pH and light intensity, water movement, stocking density, and container surface area (Hijriah 2012) [5].

According to Fitriana *et al*. (2021) [4], oxygen deficiency is one of the causes of death in developing eggs or embryos. Low water temperatures slow down the metabolic process of eggs, resulting in a longer hatching period. Low water temperatures also provide an environment conducive to fungal growth. Conversely, high temperatures accelerate the metabolic process and can even cause abnormalities and kill the eggs.

Development of Pigmentation in Pawas Fish Larvae

The results of the development of pigmentation in pawas fish larvae from each treatment can be seen in Table 3. Development of Pigmentation in Pawas Fish Larvae The results of the development of pigmentation in pawas fish larvae from each treatment can be seen in Table 3.

Table 3: Pigmentation Development of Pawas Fish Larvae

Larvae Age (hours)	Treatment	Pigmentation development
Hatching	Treatment 0	▪ Black pigmentation on the head is still minimal, mouth is still faint.
	Treatment 1	▪ Eyes are pigmented, mouth is not yet distinguishable, black spots on the head are minimal.
	Treatment 2	▪ One eye is pigmented, black spots appear on the anterior head.
	Treatment 3	▪ One eye is pigmented, black spots appear on the anterior head.
	Treatment 4	▪ One eye is pigmented, black spots appear on the anterior head.
12 Hour	Treatment 0	▪ Dark spots appear on the head, stomach, and back of the body, and eye pigmentation appears.
	Treatment 1	▪ Black spots appear on the head, stomach, and back of the body, and eye pigmentation appears.
	Treatment 2	▪ Black spots appear on the head, stomach, and back of the body, and eye pigmentation appears.
	Treatment 3	▪ Black spots appear on the head, stomach, and back of the body, but are not yet very noticeable, and eye pigmentation appears.
	Treatment 4	▪ Black spots appear on the head, stomach, and back of the body, but are not yet very noticeable.
24 Hour	Treatment 0	▪ Eye spots are complete and darkly pigmented, mouth is still closed.
	Treatment 1	▪ Eye spots are complete and darkly pigmented, and black spots are scattered on the dorsal fin.
	Treatment 2	▪ Eye spots are complete and darkly pigmented, mouth is still closed, digestive tract appears.
	Treatment 3	▪ Eye spots are complete and darkly pigmented, and black spots are scattered on the dorsal fin and body, the mouth is still closed, and the digestive tract (intestines) appears.
	Treatment 4	▪ Eye spots are complete and darkly pigmented, the mouth is still closed
36 Hour	Treatment 0	▪ Round eyes are formed with dark/black pigmentation, the yolk sac is decreasing
	Treatment 1	▪ Round eyes are formed with dark/black pigmentation, the yolk sac is decreasing, and the mouth is open.
	Treatment 2	▪ Round eyes are formed with dark/black pigmentation, the yolk sac is decreasing, and the mouth is open.
	Treatment 3	▪ Body pigments are more widely distributed in the anterior region and the yolk sac is shrinking.
	Treatment 4	▪ Body pigments are more widely distributed in the anterior region and the yolk sac is shrinking.
72 Hour	Treatment 0	▪ Denser melanophores are visible in the head region compared to the body.
	Treatment 1	▪ Denser melanophores are visible in the head region compared to the body.
	Treatment 2	▪ Denser melanophores are visible in the head region compared to the body.
	Treatment 3	▪ Denser and more numerous melanophores are visible in the head region compared to the body.
	Treatment 4	▪ Denser melanophores are visible in the head region compared to the body.
84 Hour	Treatment 0	▪ More body pigment and the egg yolk has shrunk.
	Treatment 1	▪ More body pigment and the egg yolk has shrunk.
	Treatment 2	▪ More body pigment and the egg yolk has shrunk.
	Treatment 3	▪ More body pigment and the egg yolk has shrunk.
	Treatment 4	▪ More body pigment and the egg yolk has shrunk.
99 Hour	Treatment 0	▪ Thicker and more pigment, the egg yolk is thin and almost gone
	Treatment 1	▪ Thicker and more abundant pigments, the egg yolk is thin and almost gone.
	Treatment 2	▪ Thicker and more abundant pigments, the egg yolk is thin and almost gone.
	Treatment 3	▪ Thicker and more abundant pigments, the egg yolk is thin and almost gone.
	Treatment 4	▪ Thicker and more abundant pigments, the egg yolk is thin and almost gone.

Based on Table 3, the development of larvae in pawas fish treated with pineapple, star fruit, cherry leaf extract, and papaya leaf extract solutions showed an increase compared to the control group. Significant changes in egg yolk absorption occurred in larvae treated with immersion. Organs such as the eye spot, mouth opening, and digestive tract (intestines), especially the mouth opening, developed 36 hours faster than in untreated larvae (control). The cherry leaf extract treatment required 99 hours for complete pigmentation compared to other treatments, where increasing larval age meant greater energy use because the larvae would experience better development toward organ perfection (Lalombo *et al*. 2021) [8].

Morphological Development of Pawas Fish Fins

The results of observations on the morphological development of pawas fish fins from each treatment can be seen in Table 4.

Table 4: Observations of the morphological development of pawas fish fins

Larvae Age (hours)	Treatment	Larval morphological development
0-7 Days	Treatment 0	1. The egg sac begins to shrink 2. The tail fin and pectoral fins are formed with soft fin rays 3. The mouth begins to form, the eyes develop, and the pectoral, dorsal, and tail fins begin to appear
	Treatment 1	▪ The egg sac begins to shrink ▪ The tail fin and pectoral fins form with soft fin rays ▪ The mouth begins to form, the eyes develop, the pectoral, dorsal, and tail fins begin to appear
	Treatment 2	▪ The egg sac begins to shrink ▪ The tail fin and pectoral fins are formed with soft fin rays ▪ The mouth begins to form, the eyes develop, the pectoral, dorsal, and tail fins begin to appear
	Treatment 3	▪ The egg sac begins to shrink ▪ The tail fin and pectoral fins are formed with soft fin rays ▪ The mouth begins to form, the eyes develop, the pectoral, dorsal, and tail fins begin to appear
	Treatment 4	▪ The egg sac begins to shrink ▪ The tail fin and pectoral fins form with soft fin rays ▪ The mouth begins to form, the eyes develop, the pectoral, dorsal, and tail fins begin to appear
8-14 Days	Treatment 0	▪ The soft rays of the pectoral fins, caudal fin, dorsal fin, and anal fin are harder than before ▪ The pectoral, ventral, dorsal, and caudal fins are fully formed and functional (swimming) ▪ The mouth is fully formed for eating natural food (rotifers, moina), the gills are developed, the lateral line is visible, and body coloration is beginning to appear.
	Treatment 1	▪ The soft rays of the pectoral, caudal, dorsal, and anal fins are harder than before. ▪ The pectoral, ventral, dorsal, and caudal fins are complete and functional (for swimming). ▪ The mouth is fully formed for eating natural food (rotifers, moina), the gills are developed, the lateral line is visible, and body color is beginning to appear.
	Treatment 2	▪ The soft rays of the pectoral, caudal, dorsal, and anal fins are harder than before. ▪ The pectoral, ventral, dorsal, and caudal fins are complete and functional (swimming). ▪ The mouth is fully formed for eating natural food (rotifers, moina), the gills are developed, the lateral line is beginning to appear, and the body color is beginning to emerge.
	Treatment 3	▪ The soft rays of the pectoral, caudal, dorsal, and anal fins are harder than before. ▪ The pectoral, ventral, dorsal, and caudal fins are complete and functional (swimming). ▪ The mouth is fully formed for eating natural food (rotifers, moina), the gills are developed, the lateral line is beginning to appear, and the body color is beginning to emerge.
	Treatment 4	▪ The soft rays of the pectoral fins, caudal fin, dorsal fin, and anal fin are harder than before. ▪ The pectoral, ventral, dorsal, and caudal fins are fully formed and functional (for swimming). ▪ The mouth is fully formed for eating natural food (moina), the gills are developed, the lateral line is visible, and body coloration is beginning to appear.
15-21 Days	Treatment 0	▪ All fins (pectoral, dorsal, ventral, anal, caudal) are complete and functional ▪ All organs are fully formed like an adult fish, rapid growth, more solid color.
	Treatment 1	▪ All fins (pectoral, dorsal, ventral, anal, caudal) are complete and functional ▪ All organs are fully formed like an adult fish, rapid growth, more solid color. ▪ Fins are more complete, body is longer and slimmer
	Treatment 2	▪ All fins (pectoral, dorsal, ventral, anal, caudal) ▪ are complete and functional ▪ All organs are fully formed like adult fish, rapid growth, more solid color. ▪ Fins are more complete, body is longer and slimmer
	Treatment 3	▪ All fins (pectoral, dorsal, ventral, anal, caudal) are complete and functional ▪ All organs are fully formed like adult fish, growth is rapid, color is more solid. ▪ More complete fins, longer and slimmer body
	Treatment 4	▪ All fins (pectoral, dorsal, ventral, anal, caudal) are complete and functional ▪ All organs are fully formed like an adult fish, rapid growth, more solid coloration. ▪ More complete fins, longer and slimmer body

Table 3 shows that when the larvae hatch and are 7 days old, the pectoral fins have formed with soft rays, while the dorsal, caudal, and anal fins are still fused and appear transparent. The caudal fin is flat. Dark pigmentation appears when the larvae hatch and is located on the head of the larvae. On days 8-14, the soft rays of the pectoral fins, caudal fin, dorsal fin, and anal fin harden more than before, and the pectoral, ventral, dorsal, and caudal fins become complete and functional (for swimming). On days 15-21, all fins are complete and the fish are almost like adult fish. Growth is an increase in length and weight over a period of time. According to Aidi (2009) [1], the factors influencing fish growth and development are the environment and the protein content in the feed, as protein functions to form new tissue for growth and replace damaged tissue. Feed quality significantly influences the growth rate of organisms, particularly the protein content in the feed.

Abnormalities

The results of observations of abnormalities in pawas fish for each treatment are presented in Table 5.

Table 5: Comparison between normal and abnormal pawas fish larvae, (a): normal fish, (b) abnormal in the head, (c) abnormal in the body, and (d) abnormal in the tail

Treatment	Abnormalities	Description
P0 (Control)	23 animals	Bcd
P1 (Pineapple Stalk)	18 animals	Bcd
P2 (Star Fruit)	16 animals	Bcd
P3 (Cherry Leaf)	11 animals	Cd
P4 (Papaya Leaf)	17 animals	Bcd

Fig 5 shows that the highest number of abnormalities occurred in P0 with 23 fish, and the lowest in P3 with 11 abnormal fish. Abnormalities in fish larvae can be observed from the shape of the head, body, or tail, which may be bent, the body may be shrunken or shorter than normal, and there may be differences in the behavior of the fish larvae (Mukti, 2005) [10]. Abnormalities are disorders that occur due to internal and external factors, namely genetics and disturbances in the living environment, which cause growth abnormalities in the organs and tissues of fish. Abnormalities are divided into two types: primary and secondary. Primary abnormalities are abnormalities that occur during the spermatogenesis process. Secondary abnormalities are abnormalities that occur due to environmental influences (Hedianto *et al*., 2003) [6]. According to Ismi (2006), abnormalities in the larval phase can also occur in open gills, mouth defects (short upper and/or lower mouth), and curved spine, including: iordosis (body curved upward), kyphosis (body curved downward), and scoliolysis (body appears shortened due to the spine curving upward and downward). Abnormal larvae are indicated by their smaller body size (premature), and may not survive long after 12 hours of hatching (Supriono *et al*., 2005) [15].

Survival Rate

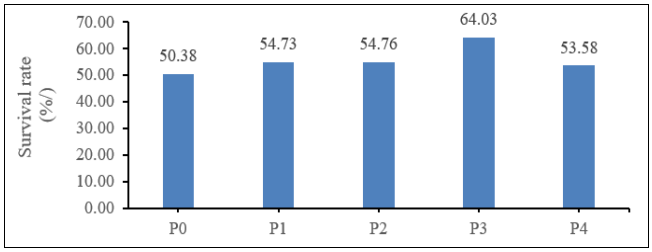

Fig 1: Survival rate of pawas fish larvae after 21 days of maintenance following egg immersion in different plant solutions

Fig 1 shows that the treatment with the highest survival rate was P3 at 64.03%, followed by P2 at 54.73%, P1 at 54.73%, P4 at 53.58%, and P0 at 50.38%. The survival rate of fish larvae is determined by a combination of various factors, including larval nutrition, environment, immunity, water quality, and feed use. Saputra *et al*. (2014) stated that the survival rate of larvae after hatching is also influenced by the quality of eggs produced by the parents. The better the egg quality, the higher the hatching rate and survival rate of the larvae. According to Mahardika *et al*. (2017) [9], survival is influenced by two factors, namely biotic and abiotic factors. The results of the analysis of variance (ANOVA) test showed that the treatment of soaking eggs with different types of natural plants had a significant effect ($P<0.05$) on the survival of pawas fish larvae. Further testing using the Student-Newman-Keuls test showed that treatments P0 and P3 were significantly different from P2 and P1 ($P<0.05$), while P4 was not significantly different ($P>0.05$) from P1 and P4.

4. Conclusion

The use of various types of natural plants affects the embryogenesis phase, hatching time, larval pigmentation development, larval fin morphology development, abnormalities, and survival rate of pawas fish larvae. The best natural plant treatment in this study was immersion in a solution of cherry leaf extract at a dose of 4 mL/L of water, resulting in an incubation period of 20 hours and 14 minutes after the embryogenesis phase, 11 abnormal larvae, and a survival rate of 64.03%.

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